

Art Unit: 1600

CLAIM'S PTO

PRE. AMDT. 09/26/01

GJT

CLAIMS 1- 7 ARE ORIGINAL

- 1                    1.    A constitutively active  
2    phosphatidylinositol 3-kinase polypeptide comprising p85  
3    subunit iSH2 domain sequences linked at the carboxy-terminus  
4    by a linker to the amino-terminus of a p110 subunit.
- 1                    2.    The polypeptide of claim 1, wherein the N-  
2    terminal 20 amino acids are eliminated from the p110 subunit.
- 1                    3.    The polypeptide of claim 1, wherein the iSH2  
2    domain sequences consist essentially of amino acids 466 to 567  
3    of the p85 subunit.
- 1                    4.    The polypeptide of claim 1 further comprising a  
2    tag at the amino or carboxy terminus.
- 1                    5.    The polypeptide of claim 4, wherein the tag is  
2    an epitope.
- 1                    6.    The polypeptide of claim 5, wherein the epitope  
2    tag is a myc epitope at the carboxy terminus of the p110  
3    subunit.
- 1                    7.    A constitutively active phosphatidylinositol 3-  
2    kinase polypeptide, comprising amino acids 466 to 567 of the  
3    p85 subunit iSH2 domain linked by a 10 amino acid glycine  
4    kinker to a p110 subunit at the amino-terminus of the p110  
5    subunit, and a myc epitope as defined by SEQ ID NO. \_\_\_\_  
6    (EQKLISEEDL) fused to the carboxy terminus of the p110  
7    subunit.

CLAIMS 8-16 ARE CANCELLED

Art Unit: 1600

## CLAIMS 17-38 ARE ORIGINAL

1           17. A method of producing an inositol phosphate  
2 product comprising reacting a phosphatidylinositol 3-kinase  
3 polypeptide of claim 1 or 7 with a phosphoinositide lipid  
4 substrate under appropriate kinase reaction conditions, and  
5 isolating the resultant product.

1           18. The method of claim 17, wherein the product is  
2 phosphatidylinositol 3'-phosphate (PI 3'-P) and the lipid  
3 substrate is phosphatidylinositol (PI).

1           19. The method of claim 17, wherein the product is  
2 phosphatidylinositol 3',4'-bisphosphate (PI 3',4'-P<sub>2</sub>) and the  
3 lipid substrate is phosphatidylinositol 4'-phosphate  
4 (PI 4'-P).

1           20. The method of claim 17, wherein the product is  
2 phosphatidylinositol 3',4',5'-phosphate (PI 3',4',5'-P<sub>3</sub>) and  
3 the lipid substrate is phosphatidylinositol 4',5'-bisphosphate  
4 (PI 4',5'-P<sub>2</sub>).

1           21. A kit for preparing an inositol phosphate  
2 product, comprising:  
3           a constitutively active phosphatidylinositol 3-  
4 kinase polypeptide of claim 1 or 7; one or more  
5 phosphoinositide substrates; and instructions for preparing  
6 the inositol phosphate reagent.

1           22. The kit of claim 21, further comprising a  
2 buffer for reconstituting said phosphatidylinositol 3-kinase  
3 polypeptide and a reaction buffer.

=00 0000000000

Art Unit: 1600

1                   23. The kit of claim 21, wherein the inositol  
2 phosphate product is selected from the group consisting of  
3 PI 3-P, PI 3',4'-P<sub>2</sub>, and PI 3',4',5'-P<sub>3</sub>.

1                   24. The kit of claim 21, wherein the  
2 phosphoinositide substrate is selected from the group  
3 consisting of PI, PI 4'-P, and PI 4',5'-P<sub>2</sub>.

25. A method of identifying a cellular target protein substrate of a  
phosphatidylinositol 3-kinase, said method comprising the steps of:  
    (a) providing a phosphatidylinositol 3-kinase polypeptide of claim 1;  
    (b) providing a test cell lysate thereof;  
    (c) providing a negative control cell lysate not contacted with said  
phosphatidylinositol 3-kinase polypeptide;  
    (d) contacting said phosphatidylinositol 3-kinase polypeptide with said test  
cell lysate in the presence of labeled ATP under conditions which allow said  
phosphatidylinositol 3-kinase to phosphorylate any cellular phosphorylated target protein; and  
    (e) comparing said test cell lysate with said negative control lysate to detect  
said phosphorylated target protein in said test cell lysate and thereby identifying the cellular  
target protein substrate.

26. The method of claim 25, wherein the ATP is [ $\gamma^{32}\text{P}$ ]ATP.

27. The method of claim 25, wherein both cell lysates are from stimulated  
cells.

28. The method of claim 25, wherein said phosphorylated target protein is detected by:

performing SDS-PAGE on said cell lysates after step (d) to separate proteins contained therein, on a gel matrix; and

preparing an autoradiograph of said gel to detect radiolabeled bands present in the test cell lysates, but absent from the negative control lysate, as indicative of the presence of said phosphorylated target protein in the test cell lysate.

29. A method of identifying an associating protein of an active phosphatidylinositol 3-kinase, comprising:

expressing a constitutively active phosphatidylinositol 3-kinase polypeptide of claim 1 or 7 in a mammalian cell under conditions which favor expression of the polypeptide;

biosynthetically labeling proteins of said active kinase expressing cell to produce labeled proteins;

obtaining a lysate from said cell;

immunoprecipitating said expressed phosphatidylinositol 3-kinase polypeptide from said cell lysate to produce an immunocomplex;

detecting a labeled protein that co-immunoprecipitates with said phosphatidylinositol 3-kinase polypeptide, within said immunocomplex, wherein said co-immunoprecipitating protein is considered an associating protein.

30. The method of claim 29, wherein said co-immunoprecipitating protein is detected by solubilizing said immunocomplex to release labeled proteins contained therein; separating said released proteins by SDS-PAGE; and performing phosphatidylinositol 3-kinase polypeptide, said labeled protein being an associating protein.

31. A method of identifying an associating protein of an active phosphatidylinositol 3-kinase, comprising;

exposing a phage or bacterial peptide library to a constitutively active phosphatidylinositol 3-kinase polypeptide of claim 1 or 7 to allow binding of a peptide from said library to said constitutively active phosphatidylinositol e-kinase polypeptide to form a bound peptide; and

isolating said bound peptide, wherein said bound peptide is considered an associating protein.

32. The method of claim 31, wherein said bound peptide is isolated by affinity purification.

33. A method of screening for an inhibitor of phosphatidylinositol 3-kinase activity, said method comprising:

providing a constitutively active phosphatidylinositol 3-kinase polypeptide of claim 1 or 7;

exposing one or more test compounds to said phosphatidylinositol 3-kinase polypeptide in the presence of a phosphatidylinositol 3-kinase substrate and [ $^{32}$ P]ATP to allow phosphorylation of said substrate; and

assaying for the presence of phosphorylated substrate wherein the absence of phosphorylated substrate is indicative that the test compound is an inhibitor of phosphatidylinositol 3-kinase activity.

34. The method of claim 33, wherein said substrate is phosphatidylinositol and said phosphorylated substrate is phosphatidylinositol 3'-P.

35. A method of treating a disease selected from the group consisting of proliferative, inflammatory, allergic and cardiovascular diseases, comprising administering to a patient, a therapeutic formulation comprising an inhibitor of phosphatidylinositol 3-kinase activity in an amount effective to block phosphatidylinositol 3-kinase activity in affected cells of said patient.

36. The method of claim 35, wherein the proliferative disease is cancer or psoriasis.

37. A method of promoting wound healing, comprising administering to a patient, a therapeutic formulation comprising a phosphatidylinositol 3-kinase polypeptide of claim 1.

38. A formulation for treating a proliferative disease, comprising a therapeutically effective amount of an inhibitor of phosphatidylinositol 3-kinase.